

What Is Claimed Is:

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said
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1. A catalytic DNA molecule having site-specific endonuclease activity specific for a nucleotide sequence defining a cleavage site in a preselected substrate nucleic acid sequence,

said molecule having first and second substrate binding regions flanking a core region,

10 wherein said first substrate binding region has a sequence complementary to a first portion of said preselected substrate nucleic acid sequence,

said second substrate binding region has a sequence complementary to a second portion of said preselected substrate nucleic acid sequence, and

15 said core region having a sequence according to the formula:

(I.) T(stem)'AGC(stem)"Z,

20 wherein said (stem)' and (stem)" are each three sequential nucleotides which when hybridized as a (stem)':(stem)" pair comprise three base pairs including at least two G:C pairs and wherein said Z = WCGR or WCGAA, and W = A or T and R = A or G; or

25 (II.) RGGCTAGCXACAAACGA (SEQ ID NO 122),

wherein said X = T, C or A, and R = A or G.

2. The molecule of claim 1 wherein said formula I defines SEQ ID NO 120 (8-17).

30 3. The molecule of claim 1 wherein said formula II defines SEQ ID NO 121 (10-23).

4. The molecule of claim 1 wherein said first or second substrate binding region is from 5 to 13 nucleotides

in length.

5. The catalytic DNA molecule of claim 1 wherein said catalytic DNA molecule comprises deoxyribonucleotides (DNA), modified DNA, nucleotide analogs, or composites thereof.

5 6. The catalytic DNA molecule of claim 1 wherein said substrate nucleic acid comprises RNA, DNA, modified RNA, modified DNA, nucleotide analogs, or composites thereof.

10 7. The catalytic DNA molecule of claim 1 wherein said catalytic DNA molecule comprises a single-stranded deoxyribonucleic acid having 5' and 3' termini, wherein said termini are modified with exonuclease-resistant nucleotides.

8. The catalytic DNA molecule of claim 7 wherein said exonuclease-resistant nucleotides comprise nucleoside phosphorothioate.

15 9. The catalytic DNA molecule of claim 1 wherein said first or second substrate binding region comprises at least two phosphorothioate nucleosides.

20 10. The catalytic DNA molecule of claim 1 wherein said core region comprises a phosphorothioate nucleoside residue on a dipyrimidine within said core.

11. The catalytic DNA molecule of claim 7 wherein said 3' termini comprises an inverted (3',3'-linked) nucleotide.

25 12. The catalytic DNA molecule of claim 1 wherein said catalytic DNA molecule comprises a 2' O-methyl ribonucleotide.

13. The catalytic DNA molecule of claim 1 wherein said first and second substrate binding regions comprise a nucleotide sequence complementary to a sequence selected from the group consisting of SEQ ID NOS 102-119.

30 14. The catalytic DNA molecule of claim 1 wherein said molecule catalyzes a reaction with a K_m of about 0.05 - 1000 nanomolar.

15. The catalytic DNA molecule of claim 1 wherein said

catalytic DNA molecule binds said substrate with a K_m of less than about 1.0 micromolar.

16. The catalytic DNA molecule of claim 1 wherein said catalytic DNA molecule binds said substrate with a K_m of about 0.1 nanomolar.

5 17. The catalytic DNA molecule of claim 1 wherein said molecule has a catalytic reaction turnover rate (k_{cat}) of about 0.005 - 0.1 min⁻¹.

10 18. The catalytic DNA molecule of claim 1 wherein said endonuclease activity is enhanced by the presence of a divalent cation.

15 19. The catalytic DNA molecule of claim 18 wherein said divalent cation is selected from the group consisting of Pb²⁺, Mg²⁺, Mn²⁺, Zn²⁺, and Ca²⁺.

20 20. The catalytic DNA molecule of claim 18 wherein said endonuclease activity is enhanced by the presence of Mg²⁺.

21. The catalytic DNA molecule of claim 1 wherein said endonuclease activity is enhanced by the presence of a monovalent cation.

25 22. The catalytic DNA molecule of claim 21, wherein said monovalent cation is selected from the group consisting of Na⁺ and K⁺.

23. A composition comprising two or more populations of catalytic DNA molecules according to claim 1, wherein each population of catalytic DNA molecules is capable of cleaving a different nucleotide sequence in a substrate.

30 24. A composition comprising two or more populations of catalytic DNA molecules according to claim 1, wherein each population of catalytic DNA molecules is capable of recognizing a different substrate.

25. A method of cleaving a target nucleic acid molecule, comprising:

a) admixing a catalytic DNA molecule according to claim 1 with a target nucleic acid molecule having a preselected substrate nucleic acid sequence to said first and second substrate binding regions, to form a reaction admixture; and

b) maintaining said admixture under predetermined reaction conditions to allow said catalytic DNA molecule to cleave said target nucleic acid molecule, thereby producing a population of substrate products.

10 26. The method of claim 25, wherein said substrate comprises RNA.

15 27. The method of claim 25, wherein said predetermined reaction conditions include the presence of a monovalent cation, a divalent cation, or both.

28. The method of claim 25 wherein said admixing comprises introducing said catalytic DNA molecule into a cell containing said target nucleic acid molecule.

20 29. A method of engineering a catalytic DNA molecule that cleaves a preselected substrate nucleic acid sequence in a target nucleic acid molecule, comprising the steps of:

a) selecting a substrate nucleic acid sequence of from 10 to 26 nucleotides in length in a target nucleic acid molecule; and

b) synthesizing a deoxyribonucleic acid molecule comprising first and second substrate binding regions flanking a core region,

25 30 wherein said first substrate binding region has a sequence complementary to a first portion of said preselected nucleic acid target sequence,

35 said second substrate binding region has a sequence

Sub X

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complementary to a second portion of said preselected nucleic acid target sequence, and

5 said core region having a sequence according to the formula:

(I.) T(stem)'AGC(stem)"Z,

10 wherein said (stem)' and (stem)" are each three sequential nucleotides which when hybridized as a (stem)':(stem)" pair comprise three base pairs including at least two G:C pairs and wherein said Z = WCGR or WCGAA, and W = A or T and R = A or G; or

(II.) RGGCTAGCXACAAACGA (SEQ ID NO 122),

15 wherein said X = T, C or A, and R = A or G.

30. The method of claim 29 wherein said formula I defines SEQ ID NO 120 (8-17).

20. The method of claim 29 wherein said formula II defines SEQ ID NO 121 (10-23).

32. The method of claim 29 wherein said first or second substrate binding region is from 5 to 13 nucleotides in length.

25. The method of claim 29 wherein said catalytic DNA molecule comprises deoxyribonucleotides (DNA), modified DNA, nucleotide analogs, or composites thereof.

34. The method of claim 29 wherein said catalytic DNA molecule comprises a single-stranded deoxyribonucleic acid having 5' and 3' termini, wherein said termini are modified 30 with exonuclease-resistant nucleotides.

35. The method of claim 7 wherein said exonuclease-resistant nucleotides comprise nucleoside phosphorothioate.

36. The method of claim 29 wherein said

first or second substrate binding region comprises at least two phosphorothioate nucleosides.

37. The method of claim 29 wherein said core region comprises a phosphorothioate nucleoside residue on a dipyrimidine within said core.

5 38. The method of claim 34 wherein said 3' termini comprises an inverted (3',3'-linked) nucleotide.

39. The method of claim 29 wherein said catalytic DNA molecule comprises a 2' O-methyl ribonucleotide.

10 40. The method of claim 29 wherein said first and second substrate binding regions comprise a nucleotide sequence complementary to a sequence selected from the group consisting of SEQ ID NOS 102-119.

15 41. The method of claim 29 wherein said molecule catalyzes a reaction with a K_m of about 0.05 - 1000 nanomolar.

42. The method of claim 29 wherein said molecule has a catalytic reaction turnover rate (k_{cat}) of about 0.005 - 0.1 min⁻¹.

20 43. The method of claim 29 wherein said endonuclease activity is enhanced by the presence of a divalent cation.

44. The method of claim 43 wherein said divalent cation is selected from the group consisting of Pb²⁺, Mg²⁺, Mn²⁺, Zn²⁺, and Ca²⁺.

25 45. The method of claim 29 wherein said endonuclease activity is enhanced by the presence of a monovalent cation.

46. The method of claim 45, wherein said monovalent cation is selected from the group consisting of Na⁺ and K⁺.